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MANGROVE ECOSYSTEMS

A MANUAL FOR THE ASSESSMENT OF BIODIVERSITY

A follow up of the
National Agricultural Technology Project
(NATP.), ICAR.

Mangrove Ecosystem Biodiversity :
Its Influence on the Natural Recruitment of
Selected Commercially Important Finfish and Shellfish
Species in Fisheries

Edited by :
Dr. George J. Parayannilam

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MANGROVES

Unique Ecosystem with rich Biodiversity, a National Wealth

1. Mangrove during High Tide.

3. *Xylocarpus granatum* with fruit -Largest Mangrove fruit.

5. *Acanthus ilicifolious* with flower.

2. Typical Mangrove Plants. *Rhizophora mucronata* in the middle flanked by *Rhizophora apiculata*

4. *Pandanus* with fruit- Leaves for mat fabrication.

Plankton - Methods for Study

K. Vijayakumaran

Collection of samples

Standard plankton net is the most commonly used apparatus for collection of plankton (IOE- Standard net has 1m^2 mouth and mesh width of 300 µm). It comprises a cone of bolting silk (or equivalent material) mounted on a ring or hoop to which are attached three thin rope bridles spliced to a small ring by means of which the net can be shackled to a towing rope or warp (Fig.1). A weight is attached to the warp to facilitate sinking of the net to the required depth and to keep horizontal opening. Horizontal or oblique haul is commonly employed though vertical haul can also be made as desired with suitable change in the attachment of weight.

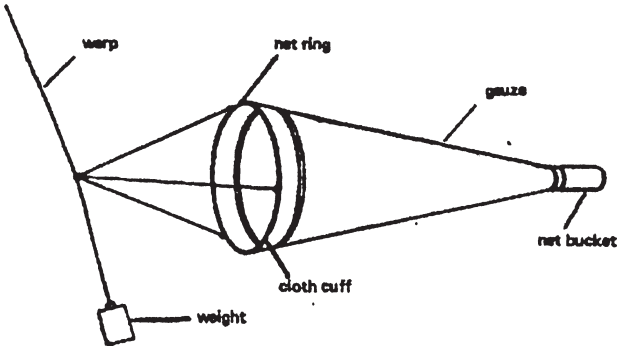


Fig. 1. Standard Net

A standard net dragged at 1-1.5 knots for ten minutes would ensure qualitative sampling. For quantitative assessment, it is very important to know the volume of water filtered through the net for assessing the quantity of plankton in unit volume. A calibrated flow meter can be attached to the mouth (a little behind the front) of the net, positioning exactly at the middle. If one digit reading of the flow meter is equal to d meter of water flow, then the volume of water filtered can be calculated at as:

Volume (m^3) of water filtered $V = \pi r^2 d \times f$

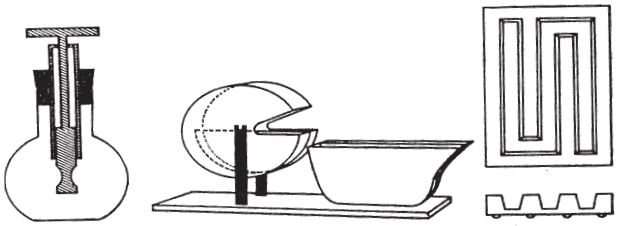
Where, ***r*** is the radius in meter of the net opening and ***f*** is the flow meter reading (final reading minus initial reading)

Preservation of sample

Although examination of live plankton has many advantages, the facilities available may not permit long storage of live specimen. Therefore, the sample must be fixed using neutral formaldehyde sufficient to bring the concentration to about 4 % in the final preserved fluid. This can be done by adding 20 ml of 40% formalin in 200-ml of water containing plankton. Use screw capped jars, preferably plastic, for storage of the preserved plankton with label carrying relevant information

Taking measurements and counts

Larger organisms (macroplankton) which are usually present in small numbers can be identified and enumerated examining with naked eye. The microplankton can be identified and enumerated only under a dissection microscope. Their numbers being far too many, the sample can be thoroughly mixed and small aliquot portions can be taken for examination and counting. Wherever possible devices such as Stempel Pipette (Fig.2) or Folsom Plankton Separator (Fig.3)can be conveniently used for accurately taking aliquots of sample and a Bogorov Counting Tray for properly counting of organisms (Fig.4). The enumerated organism must be recorded in a systematic order (see Appendix-1). Individual items difficult to identify must be kept in separate specimen tubes properly labeled for future identification. Organisms of special interest also can be separated for measurement, identification and enumeration.



2 3 4

Fig. 2. Stempel pipette, (3). Flosom plankton separator, (4) Bogorov counting tray

The volume of plankton can be determined by the displacement method. Extremely large specimens such as jellyfish have to be separated before taking measurements in order to avoid unusual values. First the total volume (ml) of the concentrated plankton plus preserving fluid (v1) is measured. Then the plankton is filtered off by suitable filter and the volume (ml) of the filtrate (v2) is measured. The final representation of plankton volume would be as:

Plankton Volume (ml m^{-3}) or = ml/m^3 =

where V is the volume of water filtered throught the net

The filtered plankton can be dried at 50^0 C in a desiccator inside an oven and then weighed rapidly. Alternatively (a more acceptable method) the fresh sample before addition of formalin can be divided into two equal portions, one portion can be preserved for subsequent sorting and counting and the other portion can be washed filtered and dried for taking weight.

Nannoplankton

The nannoplankton, which consists predominantly of small flagellates, can only be obtained quantitatively from bottle samples. Though the quantity of sample has to be decided depending up on the density of plankton, concentration techniques such as sedimentation (Utermöhl technique) can be conveniently followed.

Add 3 ml of Lugol's Iodine to one litre of the seawater sample, (see Appendix-2 for method of preparation) and allow standing for 24 hours in a measuring cylinder. After all the particulate matter had settled down to the bottom, siphon off the supernatant water taking care that the settled sediment has left undisturbed. The one litre sample may thus be reduced to about 60 ml. Use only clear glass bottles to store the sample (plastic bottles would take up iodine from the solution).

Counting

Mix thoroughly the settled sample and take aliquot (one-ml) of the sample and count using a Sedgwick-Rafter counting chamber under binocular microscope for enumeration. Qualitative and quantitative enumeration have to be done by counting replicate aliquots and the average can be taken for estimating the cell count in one litre. In the case of chain forming species, the number of chains can be counted. The cell count of different species (ni) per litre can be calculated at by the following method.

Number of cells per litre of **i^{th}** species,

Where, is the average count of ith species, 'V' the volume of sample and 'v' the volume to which the sample was reduced.

The total plankton cell count (N) per litre can be estiamted by $N = \sum_{i=1}^{s} n_i$

Haemocytometer

Alternatively, when the plankton cells are numerous and less than 30 µ in size, the cell counting can be done using a haemocytometer. The haemocytometer with an improved Neubauer ruling must be rinsed clean and dried. The face of the counting chamber is composed of two girded surfaces separated by canals. The coverslip has to be placed on the support bars along the canals. A drop of homogeneously mixed algal suspension (of 60 ml settled portion mentioned above) is delivered from a Pasteur pipette by touching the pipette tip to the edge of the cover slip where it hangs over the V-shaped loading port. Both the chambers must be loaded to seat the coverslip properly. If the algal suspension overflows either side, the chamber must be cleaned and refilled.

Each half of the haemocytometer surface contains nine large grids. Only those algal cells, which fall within the four large corner grids (numbered 1 to 4), are to be counted. Each larger corner grid is further divided into 16 small squares. Moving systematically back and forth across the squares, a minimum of 200 algal cells may be counted in as many grids as necessary. Cells falling on the border are counted if at least half the cell is within the square, taking necessary caution not to count the same cell twice. The number of algal cells per ml can be arrived at by dividing the number of cells counted by the larger corner grid area covered for counting multiplied by 10,000. For example, if one and a half large corner grids (or 24 small squares) were covered to count 300 cells, the cell density is equal to 2×10^6. In general the ultimate cell count per litre can be worked out by the formula:

$$\text{Number of cells per litre} = \frac{n}{g} \times 10000 \times \frac{v}{V}$$

Where, n is the number of algal cells counted, g the number of corner grids covered, 'V' the volume

of sample and 'v' the volume to which the sample was reduced after settlement.

Different ecological indices can be worked out using formula given in Appendix 4.

A simplified method for counting phytoplankton is 'microtransect method' (Vollenweider, 1974).

It is described by Lackey (1938) and further explained by Vollenweider, 1974 in IBP Handbook No. 12 has the great advantage, that it does not requiere specialized instruments. It is indeed capable of providing more or less precise results as those of advanced methods, if increase the number of drops/ sample enumerated.

The microtransect is the area of field of visions seen through a microscope when the slide is moved in a direction by the help of a stage meter. A path is observed comprising several field of visions from one end of a square / rectangular cover slip / glass to the other end. If a measured amount of phytoplankton sample can put under the cover glass, the transect represents a known volume of sample and number of individuals per ml can be calculated. It can be later converted into no/litre or m^3 depends on the method of sample collection.

The sample must be small to fit entirely under a cover glass. Such a quantity may be invariably one drop from a medicine dropper of ordinary diameter. Chemical volumetric pipette should not be used because of very narrow opening. The dropper must be caliberated. The sizes of drops of samples fall freely from dropper are almost uniform. The dropper should be held vertically and time to be given to from each drop in full size.

Measure the width (maximum diameter of field of vision & $\frac{d}{2}$ is the radius) of the high power field with a stage micrometer after caliberation with ocular meter. The area of one field of vision can be calculated by the formula πr^2. Every transect will give a fraction of the area under the cover slip and a definit quantity of sample.

Mix the sample thoroughly to distribute evenly the plankton before taking the drop. Then carefully deliver a drop to a clean slide and cover with an appropriate cover slip. The sample should not go beyond the margins of coverslip. One can achieve the general / uniform distribution of organisms by practice and can be checked by low power also. It is better to use the same eye piece, objective and microscope until counting of all the samples in a single programme/ project.

Move the slide, so that a transect is examined across the middle of the cover glass and count the plankton. Record and count several separate transect. Count another drop from same sample and if results are varying much, count a third drop. Subsampling or taking drop must be done very carefully.

Calculation

$$\text{Total number / drop (can be standardised as 0.1 ml)} = \frac{\text{Area of covergalss}}{\text{Area of transect}} \times \text{No. of counts / transect}$$

The value can be converted later into no./ltr. or m^2 or m^3 depends on method of sampling.

Suggested References

Hardy, A.C., 1956. *The open sea - Its natural history; the world of plankton*. New Naturalist series, Collins, London 336 p.

Lackey, J.B., 1938. The manipulation and counting of river plankton and changes in some organisms due to formalin preservation. *U.S. Public. Health Reports*, **53**, 2080-2093.

Ludwig, J.A. and J.F. Reynolds, 1988. *Statistical Ecology a Primer on Methods and Computing*. Wiley- Interscience Publication, John Wiley & Sons, Inc. New York, 337p.

Newell, G.E. and R.C. Newell, 1963. *Marine Plankton; a practical guide*. Hutchinson Educational, London, 207p.

Schlieper, C., 1972. *Research Methods in Marine Biology*. Sidgwick & Jackson, London, 356 p.

Smith, L.L, J.M. Fox, and G.D. Treece, 1993. Intensive algae culture techniques. In: McVey, J.P. (Ed), *Crustacean Aquaculture*, CRC Handbook of mariculture 2nd Edition Volume1, CRC Press, Boca Raton, Florida 33431, USA. 1-13 p.

Subrahmanyan, R., (1946) A systematic account of the marine plankton diatoms of the madras coasts. *Proc. Indian Acad. Sci*. **24** (5): 85-197.

UNESCO, 1978. *Phytoplankton Manual. Monographs on Oceanographic Methodologies*, No. **6**. UNESCO, 337p.

UNESCO, 1983. Guideline for marine biological reference collections *UNESCO Rep. Mar. Sci*. No.**22**, 63 p.

Vollenweider R. A., 1974. A Manual on Methods for Measuring Primary Production in Aquatic Environments (IBP Handbook No. **12**). Blackwell Scientific Publications Oxford. 14-16.